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Thermodynamic calculations for biochemical transport and reaction processes in metabolic networks

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Supporting Material

Authors: Stefan Jasper Jol, Anne Kümmel, Vassily Hatzimanikatis,
Daniel

Title: Thermodynamic calculations for biochemical transport and
reaction

Supplement 1. Derivation of Equations 2 and 3

Here, we translate Eq. 1 from the main text into an equation that only uses known reactant concentrations and the species' Gibbs energies of formation ($\Delta_f G_i^0$). We begin by dividing the participating species into groups. Because protons are not considered as reactants, we define the following relationship between the species:

$$_1 = \{i \in I(j), i \in \text{protons}\}, \quad (\text{A1a})$$

where $i \in I(j)$ indicates the species of reactant j , ($i \in \text{protons}$) protons that are part of the reaction. With the groups defined in Eq. A1a, we can define the following relationship between the stoichiometric coefficients of reactants and species:

$$\sum_i s_i = \sum_j \sum_{i \in I(j)} s_i + \sum_{i \in \text{protons}} s_i. \quad (\text{A1b})$$

Using Eq. A1b we can write Eq. 1 as

$$\begin{aligned} \Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j \sum_{i \in I(j)} s_i \ln c_i \\ & + \sum_{i \in \text{protons}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]. \end{aligned} \quad (\text{A1c})$$

Then we can define a relationship between reactant concentration and species concentration:

$$c_i = \alpha_{ij} \cdot C_j, \quad (\text{A1d})$$

where α_{ij} is the mol fraction ($\alpha_{ij} \in [0, 1]$) of concentration of species i to the concentration of reactant j . Using Eq. A1d and Eq. A1c we eliminate the species' concentrations:

$$\begin{aligned} \Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j \sum_{i \in I(j)} s_i \ln \alpha_{i,j} + RT \sum_j S_j \ln C_j \\ & + \sum_i \sum_{i \in \text{protons}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]. \end{aligned} \quad (\text{A1e})$$

In Eq. A1e, $C_{j,i}$ indicates the reactant concentration of reactant j that belongs to species i .

Eq. A1e may now be simplified using the concept of binding polynomials or Alberty's Legendre transform formalism.

Derivation using Alberty's concept of transformed Gibbs energies of formation

As described by Alberty (1), the ratio between a reactant concentration and one of its species concentrations, α_{ij} , can be defined with standard transformed Gibbs energies of

formation:

$$\alpha_{ij} = \frac{c_i}{C_j} = \exp\left(\frac{\Delta_f G'_j{}^0 - \Delta_f G'_i{}^0}{RT}\right), \quad (\text{A1f})$$

where $\Delta_f G'_j{}^0$ and the $\Delta_f G'_i{}^0$ are the standard transformed Gibbs energies of formation for a reactant j and a species i belonging to that reactant, respectively.

Now, we can substitute α_{ij} in Eq. A1e with Eq. A1f:

$$\begin{aligned} \Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G'_i{}^0 + \sum_j \sum_{i \in I(j)} s_i \left(\Delta_f G'_j{}^0 - \Delta_f G'_i{}^0 \right) + RT \sum_j S_j \ln C_j \\ & + \sum_{i \in \text{protons}} s_i \Delta_f G'_{\text{H}^+}{}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]. \end{aligned} \quad (\text{A1g})$$

The goal of the following derivation is to eliminate the untransformed Gibbs formation energy ($\Delta_f G'_i{}^0$) values from Eq. A1g. First we expand Eq. A1g:

$$\begin{aligned} \Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G'_i{}^0 + \sum_j S_j \Delta_f G'_j{}^0 - \sum_j \sum_{i \in I(j)} s_i \Delta_f G'_i{}^0 + RT \sum_j S_j \ln C_j \\ & + \sum_{i \in \text{protons}} s_i \Delta_f G'_{\text{H}^+}{}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]. \end{aligned} \quad (\text{A1h})$$

In order to expand the $\Delta_f G'_i{}^0$ we can define the following relation:

$$\Delta_f G'_i{}^0 = \Delta_f G'_{\text{H}^+}{}^0 - N_{\text{H},i} \left(\Delta_f G'_{\text{H}^+}{}^0 + RT \ln [\text{H}^+] \right), \quad (\text{A1i})$$

where N_{H} is the number of H atoms in species i , $\Delta_f G'_{\text{H}^+}{}^0$ is the standard Gibbs energy of formation of a hydrogen species, and $[\text{H}^+]$ is the proton concentration.

By substituting the $\Delta_f G'_i{}^0$ in Eq. A1h we obtain:

$$\begin{aligned} \Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G'_i{}^0 + \sum_j S_j \Delta_f G'_j{}^0 - \sum_j \sum_{i \in I(j)} s_i \Delta_f G'_i{}^0 + RT \sum_j S_j \ln C_j \\ & + \sum_j \sum_{i \in I(j)} s_i N_{\text{H},i} \left(\Delta_f G'_{\text{H}^+}{}^0 + RT \ln [\text{H}^+] \right) \\ & + \sum_{i \in \text{protons}} s_i \Delta_f G'_{\text{H}^+}{}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]. \end{aligned} \quad (\text{A1j})$$

Because protons are not considered as reactants, the balance of the H species in all reactants corresponds to the change in the number of free protons:

$$\sum_{i \in \text{protons}} s_i = - \sum_j \sum_{i \in I(j)} s_i N_{\text{H},i}. \quad (\text{A1k})$$

Using Eq. A1k and Eq. A1j we get

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + \sum_j S_j \Delta_f G_j'^0 - \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j S_j \ln C_j \\
& + \sum_j \sum_{i \in I(j)} s_i N_{H,i} (\Delta_f G_{H^+}^0 + RT \ln[H^+]) \\
& - \sum_j \sum_{i \in I(j)} s_i N_{H,i} \Delta_f G_{H^+}^0 - RT \sum_j \sum_{i \in I(j)} s_i N_{H,i} \ln[H^+],
\end{aligned} \tag{A1l}$$

which we can simplify by eliminating the duplicate terms with $\Delta_f G_i^0$ and $N_{H,i}$ to obtain Eq. 2 from the main text:

$$\Delta G = \sum_j S_j \Delta_f G_j'^0 + RT \sum_j S_j \ln C_j. \tag{2}$$

The derivation shown here is similar to the derivation by Alberty in his textbook in section 4.5, and arrives at the same formulation as Eq. 2 (2).

Derivation using the concept of binding polynomials

Using binding polynomials, we can define the relationship between a reactant concentration and one of its species concentration as follows (3):

$$\alpha_{ij} = \frac{c_i}{C_j} = \frac{\frac{[H^+]^i}{\prod_{k \leq i} K_k}}{\left(1 + \sum_{i=1}^N \frac{[H^+]^i}{\prod_{k \leq i} K_k}\right)} = \frac{\frac{[H^+]^i}{\prod_{k \leq i} K_k}}{P_j([H^+])}, \tag{A1l}$$

where K_k is the equilibrium constant for proton dissociation of species k (i.e., $K_k = [H^+] \cdot c_{k-1}/c_k$).

Now, we can substitute α_{ij} in Eq. A1e with Eq. A1l:

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j \sum_{i \in I(j)} s_i \ln \frac{\frac{[H^+]^i}{\prod_{k \leq i} K_k}}{P_j([H^+])} + RT \sum_j S_j \ln C_j \\
& + \sum_{i \in \text{protons}} s_i \Delta_f G_{H^+}^0 + RT \sum_{i \in \text{protons}} s_i \ln[H^+].
\end{aligned} \tag{A1m}$$

In order to simplify Eq. A1m we choose to use the reference species of each reactant for the Gibbs energy of formation, therefore we introduce $\Delta_f G_{i_{\text{ref}}}^0$, and the denominator of the expression for α_{ij} evaluates to 1. With these substitutions we obtain Eq. 3 from the

main text:

$$\begin{aligned}\Delta G = & \sum_j S_j \Delta_f G_{i_{\text{ref}}}^0 - RT \sum_j S_j \ln P_j([\text{H}^+]) + RT \sum_j S_j \ln C_j \\ & + \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+].\end{aligned}\tag{3}$$

References

1. Alberty, R. A., 1998. Calculation of Standard Transformed Gibbs Energies and Standard Transformed Enthalpies of Biochemical Reactants. *Archives of Biochemistry and Biophysics* 353:116–130.
2. Alberty, R. A., 2003. Thermodynamics of biochemical reactions. Wiley-Interscience.
3. Beard, D. A., and H. Qian, 2008. Chemical Biophysics: Quantitative Analysis of Cellular Systems. Cambridge University Press.

Supplement 2. Derivation of Equations 6 and 7

Here, we translate Eq. 4 from the main text into an equation that only uses known reactant concentrations and the (known) species' energies of formation ($\Delta_f G_i^0$). We begin by dividing the participating species into groups. Because protons are not considered as reactants, and because we want to consider the species that are transported, we define the following relationship between the species:

$$1 = \{i \in I(j), i \in \text{protons}, i \in \text{transported}\}, \quad (\text{A2a})$$

where $i \in I(j)$ indicates the species of reactant j , ($i \in \text{protons}$) that are part of the reaction and not transported, and ($i \in \text{transported}$) are all the known species that are transported. With the groups defined in Eq. A2a, we can define the following relationship between the stoichiometric coefficients of reactants and species:

$$\sum_i s_i = \sum_j \sum_{i \in I(j)} s_i + \sum_{i \in \text{protons}} s_i + \sum_{i \in \text{transported}} s_i, \quad (\text{A2b})$$

Using Eq. A2b we can write Eq. 4 as

$$\begin{aligned} \Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j \sum_{i \in I(j)} s_i \ln c_i \\ & + \sum_i \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\ & + \sum_i \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{transported}} s_i \ln c_i \\ & + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i, \end{aligned} \quad (\text{A2c})$$

where for the ($i \in \text{protons}$) part c_i is replaced by $[\text{H}^+]_i$, which is the proton concentration in its compartment, and $\Delta_f G_i^0$ is replaced by $\Delta_f G_{\text{H}^+, i}^0$, which is the Gibbs energy of formation of a proton in its specific compartment.

Then we can define a relationship between reactant concentration and species concentration:

$$c_i = \alpha_{ij} \cdot C_j, \quad (\text{A2d})$$

where α_{ij} is the mol fraction ($\alpha_{ij} \in [0, 1]$) of concentration of species i to the concentration of reactant j . Using Eq. A2d and Eq. A2c we eliminated the species' concentra-

tions:

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j \sum_{i \in I(j)} s_i \ln \alpha_{i,j} + RT \sum_j S_j \ln C_j \\
& + \sum_i \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
& + \sum_i \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{transported}} s_i \ln \alpha_{i,j} + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{A2e}
\end{aligned}$$

In Eq. A2e, $C_{j,i}$ indicates the reactant concentration of reactant j that belongs to species i .

Eq. A2e may now be simplified using the concept of binding polynomials or Alberty's Legendre transform formalism.

Derivation using Alberty's concept of transformed Gibbs energies of formation

As described by Alberty (1), the ratio between a reactant concentration and one of its species concentrations, α_{ij} , can be defined with standard transformed Gibbs energies of formation:

$$\alpha_{ij} = \frac{c_i}{C_j} = \exp \left(\frac{\Delta_f G_j'^0 - \Delta_f G_i'^0}{RT} \right), \tag{A2f}$$

where $\Delta_f G_j'^0$ and the $\Delta_f G_i'^0$ are the standard transformed Gibbs energies of formation for a reactant j and a species i belonging to that reactant, respectively.

Now, we can substitute α_{ij} in Eq. A2e with Eq. A2f:

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + \sum_j \sum_{i \in I(j)} s_i \left(\Delta_f G_j'^0 - \Delta_f G_i'^0 \right) + RT \sum_j S_j \ln C_j \\
& + \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + \sum_{i \in \text{transported}} s_i \left(\Delta_f G_j'^0 - \Delta_f G_i'^0 \right) + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{A2g}
\end{aligned}$$

The goal of the following derivation is to eliminate the untransformed Gibbs formation

energy ($\Delta_f G_i^0$) values from Eq. A2g. First we expand Eq. A2g:

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + \sum_j S_j \Delta_f G_j'^0 - \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i'^0 + RT \sum_j S_j \ln C_j \\
& + \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + \sum_{i \in \text{transported}} s_i \Delta_f G_j'^0 - \sum_{i \in \text{transported}} s_i \Delta_f G_i'^0 + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{A2h}
\end{aligned}$$

In order to expand the $\Delta_f G_i'^0$ we can define the following relation:

$$\Delta_f G_i'^0 = \Delta_f G_i^0 - N_{\text{H}, i} \left(\Delta_f G_{\text{H}^+, i}^0 + RT \ln [\text{H}^+]_i \right), \tag{A2i}$$

where N_{H} is the number of H atoms in species i , and $\Delta_f G_{\text{H}^+, i}^0$ is the standard energy of formation of a hydrogen species in the compartment of species i .

Eq. A2h now becomes:

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + \sum_j S_j \Delta_f G_j'^0 - \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j S_j \ln C_j \\
& + \sum_j \sum_{i \in I(j)} s_i N_{\text{H}, i} \left(\Delta_f G_{\text{H}^+, i}^0 + RT \ln [\text{H}^+]_i \right) \\
& + \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + \sum_{i \in \text{transported}} s_i \Delta_f G_j'^0 - \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 \\
& + \sum_{i \in \text{transported}} s_i N_{\text{H}, i} \left(\Delta_f G_{\text{H}^+, i}^0 + RT \ln [\text{H}^+]_i \right) + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{A2j}
\end{aligned}$$

Because protons are not considered as reactants, the balance of the H species in all reactants corresponds to the change in the number of free protons:

$$\sum_{i \in \text{protons}} s_i = - \sum_j \sum_{i \in I(j)} s_i N_{\text{H}, i}. \tag{A2k}$$

Using Eq. A2k and Eq. A2j we get

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + \sum_j S_j \Delta_f G_j'^0 - \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + RT \sum_j S_j \ln C_j \\
& + \sum_j \sum_{i \in I(j)} s_i N_{H,i} \left(\Delta_f G_{H^+,i}^0 + RT \ln[H^+]_i \right) \\
& - \sum_j \sum_{i \in I(j)} s_i N_{H,i} \Delta_f G_{H^+,i}^0 - RT \sum_j \sum_{i \in I(j)} s_i N_{H,i} \ln[H^+]_i \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + \sum_{i \in \text{transported}} s_i \Delta_f G_j'^0 - \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 \\
& + \sum_{i \in \text{transported}} s_i N_{H,i} \left(\Delta_f G_{H^+,i}^0 + RT \ln[H^+]_i \right) + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i, \tag{A2l}
\end{aligned}$$

which we can simplify by eliminating the duplicate terms with $\Delta_f G_i^0$ and $N_{H,i}$ to obtain Eq. 6 from the main text:

$$\begin{aligned}
\Delta G = & \sum_j S_j \Delta_f G_j'^0 + RT \sum_j S_j \ln C_j + \sum_{i \in \text{transported}} s_i \Delta_f G_j'^0 \\
& + \sum_{i \in \text{transported}} s_i N_{H,i} \left(\Delta_f G_{H^+,i}^0 + RT \ln[H^+]_i \right) + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{6}
\end{aligned}$$

Derivation using the concept of binding polynomials

Using binding polynomials, we can define the relationship between a reactant concentration and one of its species concentration as follows (2):

$$\alpha_{ij} = \frac{c_i}{C_j} = \frac{\frac{[H^+]^i}{\prod_{k \leq i} K_k}}{\left(1 + \sum_{i=1}^N \frac{[H^+]^i}{\prod_{k \leq i} K_k} \right)} = \frac{\frac{[H^+]^i}{\prod_{k \leq i} K_k}}{P_j([H^+])}, \tag{A2l}$$

where K_k is the equilibrium constant for proton dissociation of species k (i.e., $K_k = [H^+] \cdot c_{k-1}/c_k$).

Now, we can substitute α_{ij} in Eq. A2e with Eq. A2l:

$$\begin{aligned}
\Delta G = & \sum_j \sum_{i \in I(j)} s_i \Delta_f G_i^0 + \sum_j \sum_{i \in I(j)} s_i \ln \frac{[\text{H}^+]^i}{P_j([\text{H}^+])} + RT \sum_j S_j \ln C_j \\
& + \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{transported}} s_i \ln \frac{[\text{H}^+]^i}{P_j([\text{H}^+])} + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{A2m}
\end{aligned}$$

In order to simplify Eq. A2m we choose to use the reference species of each reactant for the Gibbs energy of formation, therefore we introduce $\Delta_f G_{i_{\text{ref}}}^0$, and the denominator of the expression for α_{ij} evaluates to 1. This substitution cannot be made for the ($i \in \text{transported}$) part, where we consider a specifically transported species. With these substitutions we obtain Eq. 7 from the main text:

$$\begin{aligned}
\Delta G = & \sum_j S_j \Delta_f G_{i_{\text{ref}}}^0 - RT \sum_j S_j \ln P_j([\text{H}^+]) + RT \sum_j S_j \ln C_j \\
& + \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{transported}} s_i \ln \frac{[\text{H}^+]^i}{P_j([\text{H}^+])} + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \tag{7}
\end{aligned}$$

References

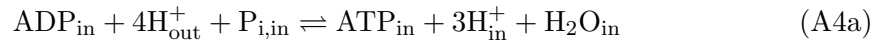
1. Alberty, R. A., 1998. Calculation of Standard Transformed Gibbs Energies and Standard Transformed Enthalpies of Biochemical Reactants. *Archives of Biochemistry and Biophysics* 353:116–130.
2. Beard, D. A., and H. Qian, 2008. Chemical Biophysics: Quantitative Analysis of Cellular Systems. Cambridge University Press.

Supplement 3. Examples and Data Sources

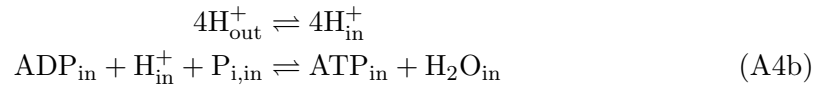
With the derived two formulas we can calculate the change in Gibbs energy of various biochemical transport and/or reaction processes. This supplement treats two examples of a transport/reaction process to make clear how to make use of the two formulations. We also present two tables with an overview of the data used in the examples that are shown in the main text. In the last two sections we show where to obtain necessary data that we use in the equations and how to correct the calculations for ionic strength.

ATP synthase

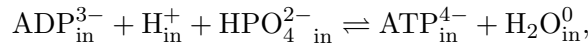
The process of generating ATP in a cell can be done by the ATP synthase complex where ADP is converted into ATP by using the proton gradient over the mitochondrial membrane. The overall biochemical equation of this process can be written as:



Equation (A4a) contains both reactants (ADP, P_{i} , ATP and H_2O) and species (H^+). In this case the protons are transported, while the reactants are participating in a biochemical reaction that takes place in a single compartment (in). We can break up equation (A4a) into two separate reactions:



If we would know the concentrations of each of the separate species, we could calculate the ΔG by generating a balanced reaction equation for the reaction part of Eq. A4b in terms of chemical species:



and using Eq. 4 from the main text:

$$\Delta G = \sum_i s_i \Delta_f G_i^0 + RT \sum_i s_i \ln c_i + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i. \quad (\text{A4c})$$

For our example we have all the species specific data given in Tab. 1. Using these data we can make the following calculation:

$$\begin{aligned} \Delta G &= -1 \cdot -1906.13 - 4 \cdot 0 - 1 \cdot 0 - 1 \cdot -1096.10 + 1 \cdot -2768.10 + 4 \cdot 0 + 1 \cdot -237.19 \\ &\quad + RT \ln \left(\frac{2.00 \times 10^{-4} \cdot (1.00 \times 10^{-7})^4 \cdot 1}{1.99 \times 10^{-4} \cdot (3.16 \times 10^{-7})^4 \cdot 1.00 \times 10^{-7} \cdot 3.77 \times 10^{-3}} \right) \\ &\quad + F \cdot -1.80 \times 10^{-1} \cdot 4 \cdot +1 \\ &= -30.15 \text{ kJ mol}^{-1} \end{aligned} \quad (\text{A4d})$$

Table 1: Species data for ATP synthase reaction.

| Species | $\Delta_f G^0$ | c_i (M) | | | |
|---|----------------|-----------------------|------------------|------------------------|--------------------------------------|
| ATP ⁴⁻ | -2768.10 | 2.00×10^{-4} | I _{in} | 0 | M |
| HATP ³⁻ | -2811.48 | 7.96×10^{-4} | I _{out} | 0 | M |
| H ₂ ATP ²⁻ | -2838.18 | 3.79×10^{-6} | T | 298.15 | K |
| ADP ³⁻ | -1906.13 | 1.99×10^{-4} | F | 96.5 | kC mol ⁻¹ |
| HADP ²⁻ | -1947.10 | 3.00×10^{-4} | φ_m | -1.80×10^{-1} | V |
| H ₂ ADP ⁻ | -1971.98 | 6.85×10^{-7} | R | 8.31×10^{-3} | kJ K ⁻¹ mol ⁻¹ |
| HPO ₄ ²⁻ | -1096.10 | 3.77×10^{-3} | | | |
| H ₂ PO ₄ ⁻ | -1137.30 | 6.23×10^{-3} | | | |
| H ₂ O ⁰ | -237.19 | 1.00 | | | |
| H _{in} ⁺ | 0.00 | 1.00×10^{-7} | | | |
| H _{out} ⁺ | 0.00 | 3.16×10^{-7} | | | |

In order to calculate the ΔG of this process based on reactant concentrations we will use the formulations that we proposed in the main text. From the two separate reactions in Eq. A4b we can define the different reactants and species that need to be considered in the separate parts of the equations to calculate the ΔG :

$$\begin{aligned}
\sum_{i \in \text{transported}} & : -4\text{H}_{\text{out}}^+ + 4\text{H}_{\text{in}}^+ \\
\sum_j & : -\text{ADP}_{\text{in}}, -\text{P}_{\text{i, in}}, +\text{ATP}_{\text{in}}, +\text{H}_2\text{O}_{\text{in}} \\
\sum_{i \in \text{protons}} & : -\text{H}_{\text{in}}^+
\end{aligned} \tag{A4e}$$

In order to calculate the ΔG of this process we need information on the concentrations of the involved reactants, the pH and ionic strength in the involved compartments and the Gibbs energies of formation of each of the involved species. This data is summarized in Table 2.

Now we can apply the two different equations (Eq. 6 and Eq. 7 from the main text) to calculate the ΔG of this reaction.

Using Alberty's Legendre transform formalism

In order to make use of the Alberty formulation we first need to calculate the standard transformed Gibbs energies of formation for *species* and *reactants*. Note that for simplicity we assume the ionic strength (I) to be zero in this example. To incorporate ionic strength we can correct $\Delta_f G_i^0$ using Eq. A4y. Also note that a measured pH incorporates the ionic strength and temperature. Therefore when calculating the $[\text{H}^+]$ by using

Table 2: Overview of input data required for ΔG calculation of ATP synthase

| Parameter | Value | Unit | Parameter | Value | Unit |
|--|----------|----------------------|------------------------------------|-------|------|
| $\Delta_f G_{\text{ADP}^{3-}}^0$ | -1906.13 | kJ mol^{-1} | $C_{\text{ATP},\text{in}}$ | 1 | mM |
| $\Delta_f G_{\text{HADP}^{2-}}^0$ | -1947.10 | kJ mol^{-1} | $C_{\text{ADP},\text{in}}$ | 0.5 | mM |
| $\Delta_f G_{\text{H}_2\text{ADP}^-}^0$ | -1971.98 | kJ mol^{-1} | $C_{\text{P}_i,\text{in}}$ | 10 | mM |
| $\Delta_f G_{\text{ATP}^{4-}}^0$ | -2768.10 | kJ mol^{-1} | $C_{\text{H}_2\text{O},\text{in}}$ | 1000 | mM |
| $\Delta_f G_{\text{HATP}^{3-}}^0$ | -2811.48 | kJ mol^{-1} | $\text{pK}_{\text{ADP},1}$ | 7.18 | - |
| $\Delta_f G_{\text{H}_2\text{ATP}^{2-}}^0$ | -2838.18 | kJ mol^{-1} | $\text{pK}_{\text{ADP},2}$ | 4.36 | - |
| $\Delta_f G_{\text{HPO}_4^{2-}}^0$ | -1069.10 | kJ mol^{-1} | $\text{pK}_{\text{ATP},1}$ | 7.60 | - |
| $\Delta_f G_{\text{H}_2\text{PO}_4^-}^0$ | -1137.30 | kJ mol^{-1} | $\text{pK}_{\text{ATP},2}$ | 4.68 | - |
| $\Delta_f G_{\text{H}_2\text{O}^0}^0$ | -237.19 | kJ mol^{-1} | $\text{pK}_{\text{P}_i,1}$ | 7.22 | - |
| $\Delta_f G_{\text{H}^+}^0$ | 0.0 | kJ mol^{-1} | pH_{in} | 7 | - |
| | | | pH_{out} | 6.5 | - |
| | | | I_{in} | 0 | M |
| | | | I_{out} | 0 | M |

$[\text{H}^+] = 10^{-\text{pH}}$, the $\Delta_f G_{\text{H}^+}^0 = 0$. To calculate the real proton concentration, we can use the following equation:

$$[\text{H}^+] = \exp \left(\frac{-\Delta_f G_{\text{H}^+}^0(I)}{RT} + \ln(10^{-\text{pH}}) \right).$$

In this example we have an ionic strength of $I = 0$, therefore we can use the pH directly to calculate the proton concentrations. We can calculate the standard transformed Gibbs energies using the following equation:

$$\Delta_f G_i'^0 = \Delta_f G_i^0 - N_{\text{H}} (\Delta_f G_{\text{H}^+}^0 + RT \ln(10^{-\text{pH}})) \quad (\text{A4f})$$

Using the numbers from Table 2, we can calculate the following values:

$$\begin{aligned}
\Delta_f G_{\text{ADP}_{\text{in}}^{3-}}'^0 &= -1906.13 - 12 \cdot (0 + RT \ln(10^{-7})) = -1426.65, \\
\Delta_f G_{\text{HADP}_{\text{in}}^{2-}}'^0 &= -1427.67, \quad \Delta_f G_{\text{H}_2\text{ADP}_{\text{in}}^-}'^0 = -1412.59, \\
\Delta_f G_{\text{ATP}_{\text{in}}^{4-}}'^0 &= -2288.62, \quad \Delta_f G_{\text{HATP}_{\text{in}}^{3-}}'^0 = -2292.05, \\
\Delta_f G_{\text{H}_2\text{ATP}_{\text{in}}^{2-}}'^0 &= -2278.79, \quad \Delta_f G_{\text{HPO}_4^{2-}_{\text{in}}}'^0 = -1056.14, \\
\Delta_f G_{\text{H}_2\text{PO}_4^-_{\text{in}}}'^0 &= -1057.39, \quad \Delta_f G_{\text{H}_2\text{O}_{\text{in}}^0}'^0 = -157.28, \\
\Delta_f G_{\text{H}^+_{\text{in}}}'^0 &= 39.96, \quad \Delta_f G_{\text{H}^+_{\text{out}}}'^0 = 37.10.
\end{aligned} \quad (\text{A4g})$$

From the $\Delta_f G_i'^0$ values calculated in (A4s) we can now calculate the $\Delta_f G_j'^0$ values for

each reactant using the following equation:

$$\Delta_f G_j^0 = -RT \ln \sum_i \exp \left(\frac{-\Delta_f G_i^0([H^+])}{RT} \right). \quad (\text{A4h})$$

Using the numbers from Table 2, we can calculate the following values:

$$\begin{aligned} \Delta_f G_{\text{ADP}}^0 &= -RT \ln \left(\exp \left(\frac{1460.90}{RT} \right) + \exp \left(\frac{1464.77}{RT} \right) + \exp \left(\frac{1452.55}{RT} \right) \right) = -1465.26 \\ \Delta_f G_{\text{ATP}}^0 &= -2329.37, \quad \Delta_f G_{\text{P}_i}^0 = -1063.53, \quad \Delta_f G_{\text{H}_2\text{O}}^0 = -162.99, \\ \Delta_f G_{\text{H}^+_{\text{in}}}^0 &= 39.96, \quad \Delta_f G_{\text{H}^+_{\text{out}}}^0 = 37.10. \end{aligned} \quad (\text{A4i})$$

When we use Eq. 6 from the main text we can fill in the separate parts:

$$\begin{aligned} \Delta G &= RT \sum_j S_j \ln C_j \\ &\quad + \sum_j S_j \Delta_f G_j^0 \\ &\quad + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\ &\quad + \sum_{i \in \text{transported}} s_i \Delta_f G_j^0 + \sum_{i \in \text{transported}} s_i N_{\text{H},i} (\Delta_f G_{\text{H}^+}^0 + RT \ln [H^+]_i) \\ &\quad + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i \\ &= RT (-1 \ln 5 \times 10^{-4} - 1 \ln 1 \times 10^{-2} + 1 \ln 1 \times 10^{-3} + 1 \ln 1) \\ &\quad + (-1 \cdot -1465.26 - 1 \cdot -1063.53 + 1 \cdot -2329.37 + 1 \cdot -162.99) \\ &\quad + RT (-4 \cdot 10^{-6.5} + 4 \cdot 10^{-7}) \\ &\quad + (-4 \cdot 37.10 + 4 \cdot 39.96) + (-4 \cdot 1 \cdot (0 + RT \ln 10^{-6.5}) + 4 \cdot 1 \cdot (0 + RT \ln 10^{-7})) \\ &\quad + F \Delta \varphi_m 4 \cdot 1 \\ &= -30.15 \text{ kJ mol}^{-1} \end{aligned} \quad (\text{A4j})$$

Using the concept of binding polynomials

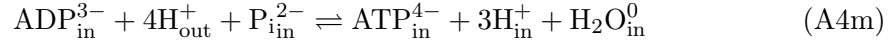
To make use of the binding polynomial formulation we first need to calculate the binding polynomials. The binding polynomial is calculated with the following equation:

$$P_j([H^+]) = 1 + \sum_{i=1}^N \frac{[H^+]^i}{\prod_{k \leq i} K_k} \quad (\text{A4k})$$

The calculation of the binding polynomials for all the involved reactants is as follows. Here we calculate the proton concentration ($[H^+]$) from the pH:

$$\begin{aligned}
P_{ADP,in} &= 1 + \frac{10^{-pH_{in} \cdot 1}}{K_{ADP,1}} + \frac{10^{-pH_{in} \cdot 2}}{K_{ADP,1} \cdot K_{ADP,2}} = 2.51 \\
P_{ATP,in} &= 1 + \frac{10^{-pH_{in} \cdot 1}}{K_{ATP,1}} + \frac{10^{-pH_{in} \cdot 2}}{K_{ATP,1} \cdot K_{ATP,2}} = 5.00 \\
P_{P_i,in} &= 1 + \frac{10^{-pH_{in} \cdot 1}}{K_{P_i,1}} = 2.65 \\
P_{H_2O,in} &= 1 \\
P_{H^+,in} &= 1 \\
P_{H^+,out} &= 1
\end{aligned} \tag{A4l}$$

Having calculated the binding polynomials, we must define the reaction (Eq. A4a) in terms of reference species, the most unbound species for each reactant:



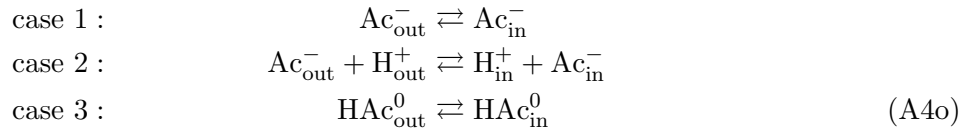
Now we fill in the equation to calculate the ΔG of the reaction based on binding polynomials. Here, the $\Delta_f G_{i_{ref}}^0$ is the $\Delta_f G^0$ of the most unbound species for each reactant

in Table 2.

$$\begin{aligned}
\Delta G &= \sum_j S_j \Delta_f G_{i_{\text{ref}}}^0 \\
&+ RT \sum_j S_j \ln C_j \\
&- RT \sum_j s_j \ln P_j([H^+]) \\
&+ \sum_{i \in \text{protons}} s_i \Delta_f G_{H^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [H^+]_i \\
&+ \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{transported}} s_i \ln C_{j, i} \\
&- RT \sum_{i \in \text{transported}} s_i \ln \frac{P_j([H^+])}{\frac{[H^+]^i}{\prod_{k \leq i} K_k}} \\
&+ F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i \\
&= (-1 \cdot -1906.13 - 1 \cdot -1069.10 + 1 \cdot -2768.10 + 1 \cdot -237.19) \\
&+ RT (-1 \ln 5 \times 10^{-4} - 1 \ln 1 \times 10^{-2} + 1 \ln 1 \times 10^{-3} + 1 \ln 1) \\
&- RT (-1 \ln 2.51 - 1 \ln 2.65 + 1 \ln 5.00 + 1 \ln 1) \\
&+ (-1 \cdot 0) + (-1 RT \ln 10^{-7}) \\
&+ (-4 \cdot 0 + 4 \cdot 0) + RT (-4 \ln 10^{-6.5} + 4 \ln 10^{-7}) \\
&- RT \left(-4 \ln \frac{1}{\frac{1}{1}} + 4 \ln \frac{1}{\frac{1}{1}} \right) \\
&+ F \Delta \varphi_m \cdot 4 \cdot 1 \\
&= -30.15 \text{ kJ mol}^{-1}
\end{aligned} \tag{A4n}$$

Acetate transport

As a second example, we consider acetate transport over a biological membrane. Because acetate can be protonated it is a reactant with two species, Ac^- and HAc^0 . We will consider the following three cases of transport of acetate:

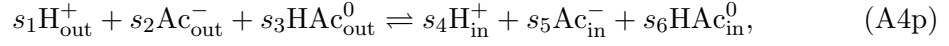


From the reaction equations in A4o we can define a general reaction equation with the

Table 3: Overview of input data required for ΔG calculation of acetate transport.

| Parameter | Value | Unit | Parameter | Value | Unit |
|--|---------|----------------------|--------------------------------------|-------|----------------------|
| $\Delta_f G_{\text{Ac}_{\text{in}}^-}^0$ | -369.31 | kJ mol ⁻¹ | $C_{\text{Ac},\text{in}}$ | 1 | mmol L ⁻¹ |
| $\Delta_f G_{\text{HAc}_{\text{in}}^0}^0$ | -396.45 | kJ mol ⁻¹ | $C_{\text{Ac},\text{out}}$ | 10 | mmol L ⁻¹ |
| $\Delta_f G_{\text{Ac}_{\text{out}}^-}^0$ | -369.31 | kJ mol ⁻¹ | $\text{pK}_{\text{Ac}_{\text{in}}}$ | 4.75 | - |
| $\Delta_f G_{\text{HAc}_{\text{out}}^0}^0$ | -396.45 | kJ mol ⁻¹ | $\text{pK}_{\text{Ac}_{\text{out}}}$ | 4.75 | - |
| $\Delta_f G_{\text{H}_{\text{in}}^+}^0$ | 0 | kJ mol ⁻¹ | pH_{in} | 7 | - |
| $\Delta_f G_{\text{H}_{\text{out}}^+}^0$ | 0 | kJ mol ⁻¹ | pH_{out} | 5 | - |
| I_{in} | 0 | | | | |
| I_{out} | 0 | | | | |

stoichiometric coefficients set as variables:



where we can define the stoichiometric matrix S with the stoichiometric coefficients in the row and the three different cases in the columns:

$$S = \begin{bmatrix} 0 & -1 & 0 \\ -1 & -1 & 0 \\ 0 & 0 & -1 \\ 0 & +1 & 0 \\ +1 & +1 & 0 \\ 0 & 0 & +1 \end{bmatrix}, \quad (\text{A4q})$$

From reaction Eq. A4p we can directly define the reaction parts we want to consider in calculating the ΔG :

$$\begin{aligned} \sum_{i \in \text{transported}} &: -s_1 \text{Ac}_{\text{out}}^-, -s_2 \text{Ac}_{\text{out}}^-, -s_3 \text{H}_{\text{out}}^+, +s_4 \text{Ac}_{\text{in}}^-, +s_5 \text{Ac}_{\text{in}}^-, +s_6 \text{H}_{\text{in}}^+ \\ \sum_j &: 0 \\ \sum_{i \in \text{protons}} &: 0 \end{aligned} \quad (\text{A4r})$$

In Table 3 we defined the necessary data to use the binding polynomial and Alberty equations.

Using Alberty's Legendre transform formalism

We first need to calculate the standard transformed Gibbs energies of formation for *species* and *reactants*. We can calculate the standard transformed Gibbs energies using

Eq. 12 from the main text as follows:

$$\begin{aligned}
\Delta_f G'^0_{\text{Ac}^-, \text{in}} &= \Delta_f G^0_{\text{Ac}^-, \text{in}} - N_{\text{H,Ac}^-, \text{in}} \left(\Delta_f G^0_{\text{H}^+, \text{in}} + RT \ln(10^{-\text{pH}_{\text{in}}}) \right) \\
&= -369.31 - 3 \cdot (0 + RT \ln(10^{-7})) = -249.44, \\
\Delta_f G'^0_{\text{HAc}^0, \text{in}} &= -236.62, \quad \Delta_f G'^0_{\text{Ac}^-, \text{out}} = -283.69, \\
\Delta_f G'^0_{\text{HAc}^0, \text{out}} &= -282.29, \quad \Delta_f G'^0_{\text{H}^+, \text{in}} = 34.25, \quad \Delta_f G'^0_{\text{H}^+, \text{out}} = 28.54. \tag{A4s}
\end{aligned}$$

From the $\Delta_f G'^0_i$ values calculated in (A4s) we can now calculate the $\Delta_f G'^0_j$ values for each reactant using Eq. 13 from the main text:

$$\begin{aligned}
\Delta_f G'^0_{\text{Ac}, \text{in}} &= -RT \ln \left(\exp \left(\frac{-\Delta_f G'^0_{\text{Ac}^-, \text{in}}}{RT} \right) + \exp \left(\frac{-\Delta_f G'^0_{\text{HAc}^0, \text{in}}}{RT} \right) \right) \\
&= -RT \ln \left(\exp \left(\frac{249.44}{RT} \right) + \exp \left(\frac{236.62}{RT} \right) \right) = -249.46 \\
\Delta_f G'^0_{\text{Ac}, \text{out}} &= -284.81, \quad \Delta_f G'^0_{\text{H}^+, \text{in}} = 34.25, \quad \Delta_f G'^0_{\text{H}^+, \text{out}} = 28.54. \tag{A4t}
\end{aligned}$$

When we use Eq. 20 from the main text we can fill in the separate parts based on the

separate parts as defined in Eq. A4r:

$$\begin{aligned}
\Delta G = & RT \sum_j S_j \ln C_j \\
& + \sum_j S_j \Delta_f G_j'^0 \\
& + RT \sum_{i \in \text{transported}} s_i \ln C_{j,i} \\
& + \sum_{i \in \text{transported}} s_i \Delta_f G_j'^0 \\
& + \sum_{i \in \text{transported}} s_i N_{H,i} \left(\Delta_f G_{H^+,i}^0 + RT \ln [H^+]_i \right) \\
& + F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i \\
= & 0 \\
& + 0 \\
& + RT (s_1 \ln 10^{-5} + s_2 \ln 10 \times 10^{-3} + s_3 \ln 10 \times 10^{-3} \\
& \quad + s_4 \ln 10^{-7} + s_5 \ln 1 \times 10^{-3} + s_6 \ln 1 \times 10^{-3}) \\
& + s_1 \cdot 28.54 + s_2 \cdot -284.81 + s_3 \cdot -284.81 \\
& \quad + s_4 \cdot 34.25 + s_5 \cdot -249.46 + s_6 \cdot -249.46 \\
& + (s_1 \cdot 1 + s_2 \cdot 3 + s_3 \cdot 4) \cdot (0 + RT \ln 10^{-5}) \\
& \quad + (s_4 \cdot 1 + s_5 \cdot 3 + s_6 \cdot 4) \cdot (0 + RT \ln 10^{-7}) \\
& + 0
\end{aligned} \tag{A4u}$$

The outcomes of Eq. A4u in each of the cases as described in reaction Eq. A4o (i.e. using the stoichiometric coefficients of the matrix S defined in (A4q)) are 12.76, -16.02 and -16.02 kJ mol⁻¹ for cases 1, 2 and 3 respectively.

Using the concept of binding polynomials

When we use the binding polynomial formulation we first need to calculate the binding polynomials. The binding polynomial is calculated using the following equation (see Eq. 10 from the main text):

$$P_j([H^+]) = 1 + \sum_{i=1}^N \frac{[H^+]^i}{\prod_{k \leq i} K_k} \tag{A4v}$$

The calculation of the binding polynomials for all the involved reactants is as follows. Here we calculate the proton concentration ($[H^+]$) from the pH:

$$\begin{aligned}
P_{Ac,in} &= 1 + \frac{10^{-pH_{in} \cdot 1}}{K_{Ac,in}} = 1.01 \\
P_{Ac,out} &= 1 + \frac{10^{-pH_{in} \cdot 1}}{K_{Ac,out}} = 1.57 \\
P_{H^+,in} &= 1 \\
P_{H^+,out} &= 1
\end{aligned} \tag{A4w}$$

Since we do not have any reactant in the reactions in Eq. A4o we do not need to define reference species. Now we fill in the equation to calculate the ΔG of the reaction based

on binding polynomials (Eq. 19 in the main text).

$$\begin{aligned}
\Delta G &= \sum_j S_j \Delta_f G_{i_{\text{ref}}}^0 \\
&+ RT \sum_j S_j \ln C_j \\
&- RT \sum_j S_j \ln P_j([\text{H}^+]) \\
&+ \sum_{i \in \text{protons}} s_i \Delta_f G_{\text{H}^+, i}^0 + RT \sum_{i \in \text{protons}} s_i \ln [\text{H}^+]_i \\
&+ \sum_{i \in \text{transported}} s_i \Delta_f G_i^0 + RT \sum_{i \in \text{transported}} s_i \ln C_{j, i} \\
&- RT \sum_{i \in \text{transported}} s_i \ln \frac{P_j([\text{H}^+])}{\frac{[\text{H}^+]^i}{\prod_{k \leq i} K_k}} \\
&+ F \Delta \varphi_m \sum_{i \in \text{inside}} s_i z_i \\
&= 0 \\
&+ 0 \\
&- 0 \\
&+ 0 \\
&+ \left(s_1 \cdot 0 + (s_2 + s_3) \cdot -369.31 + s_4 \cdot 0 + (s_5 + s_6) \cdot -369.31 \right) \\
&\quad + RT \left(s_1 \ln 10^{-5} + (s_2 + s_3) \ln 10 \times 10^{-3} + s_4 \ln 10^{-7} + (s_5 + s_6) \ln 1 \times 10^{-3} \right) \\
&- RT \left(s_1 \ln \frac{1}{\frac{1}{1}} + s_2 \ln \frac{1.57}{\frac{1}{1}} + s_3 \ln \frac{1.57}{\left(\frac{10^{-5.1}}{4.75} \right)} \right. \\
&\quad \left. + s_4 \ln \frac{1}{\frac{1}{1}} + s_5 \ln \frac{1.01}{\frac{1}{1}} + s_6 \ln \frac{1.01}{\left(\frac{10^{-7.1}}{4.75} \right)} \right) \\
&+ F \Delta \varphi_m \cdot (s_4 \cdot 1 + s_5 \cdot -1) \tag{A4x}
\end{aligned}$$

The outcomes of Eq. A4x in each of the cases as described in reaction Eq. A4o (i.e. using the stoichiometric coefficients of the matrix S defined in (A4q)) are 12.76, -16.02 and -16.02 kJ mol⁻¹ for cases 1, 2 and 3 respectively.

Supplementary information for the examples in the main text

Tab. 4 shows additional values next to Tab. 1 from the main text for the example calculation of Succinate transport. In Tab. 5 additional values for the example calculation on ATP synthase is shown.

Table 4: Values as used in the example calculation of ATP synthase. Gibbs energies of formation are in kJ mol^{-1} . Please note that the transformed Gibbs energies of formation ($\Delta_f G_i^0$ and $\Delta_f G_j^0$) are specific for the indicated pH.

| Species | $\Delta_f G_i^0$ | z | N_H | $\Delta_f G_i^0(I)$ | $\Delta_f G_i^0$ |
|-----------------------------------|------------------|-------|-------------------|------------------------|------------------------------------|
| Succ _{in} ²⁻ | -690.44 | -2 | 4 | -693.23 | -542.03 |
| Succ _{out} ²⁻ | -690.44 | -2 | 4 | -693.23 | -576.28 |
| Succ _{in} ⁻ | -722.62 | -1 | 5 | -723.32 | -534.32 |
| Succ _{out} ⁻ | -722.62 | -1 | 5 | -723.32 | -577.13 |
| Succ _{in} ⁰ | -746.64 | 0 | 6 | -746.64 | -519.84 |
| Succ _{out} ⁰ | -746.64 | 0 | 6 | -746.64 | -571.22 |
| H _{in} ⁺ | 0.00 | 1 | 1 | -0.697 | 37.10 |
| H _{out} ⁺ | 0.00 | 1 | 1 | -0.697 | 28.54 |
| Reactant | $\Delta_f G_j^0$ | P_j | Other | | |
| Succ _{in} | -542.15 | 1.04 | I_{in} | 0.15 | M |
| Succ _{out} | -578.59 | 2.54 | I_{out} | 0.15 | M |
| H _{in} ⁺ | 37.10 | 1 | pH _{in} | 6.5 | |
| H _{out} ⁺ | 28.54 | 1 | pH _{out} | 5 | |
| | | | φ_m | -180 | mV |
| | | | R | 8.314×10^{-3} | $\text{kJ K}^{-1} \text{mol}^{-1}$ |
| | | | T | 298.15 | K |
| | | | F | 96.49 | kC mol^{-1} |

Table 5: Values as used in the example calculation of ATP synthase. Gibbs energies of formation are in kJ mol^{-1} . Please note that the transformed Gibbs energies of formation ($\Delta_f G_i^0$ and $\Delta_f G_j^0$) are specific for the indicated pH.

| Species | $\Delta_f G_i^0$ | z | N_H | $\Delta_f G_i^0(I)$ | $\Delta_f G_i^0$ |
|-------------------------------|------------------|-------|-------------------|------------------------|------------------------------------|
| ADP ³⁻ | -1906.13 | -3 | 12 | -1912.40 | -1424.56 |
| ADP ²⁻ | -1947.10 | -2 | 13 | -1949.89 | -1421.40 |
| ADP ⁻ | -1971.98 | -1 | 14 | -1972.68 | -1403.53 |
| ATP ⁴⁻ | -2768.10 | -4 | 12 | -2779.25 | -2291.41 |
| ATP ³⁻ | -2811.48 | -3 | 13 | -2817.75 | -2289.26 |
| ATP ²⁻ | -2838.18 | -2 | 14 | -2840.97 | -2271.82 |
| Pi ²⁻ | -1096.10 | -2 | 1 | -1098.89 | -1058.23 |
| Pi ⁻ | -1137.30 | -1 | 2 | -1138.00 | -1056.69 |
| H ₂ O ⁰ | -237.19 | 0 | 2 | -237.19 | -155.88 |
| H _{in} ⁺ | 0.00 | 1 | 1 | -0.697 | 39.96 |
| H _{out} ⁺ | 0.00 | 1 | 1 | -0.697 | 37.10 |
| Reactant | $\Delta_f G_j^0$ | P_j | Other | | |
| ADP | -1425.17 | 1.28 | I_{in} | 0.15 | M |
| ATP | -2292.28 | 1.42 | I_{out} | 0.15 | M |
| P _i | -1059.30 | 1.54 | pH _{in} | 7 | |
| H ₂ O | -155.88 | 1 | pH _{out} | 6.5 | |
| H _{in} ⁺ | 39.96 | 1 | φ_m | 40 | mV |
| H _{out} ⁺ | 37.10 | 1 | R | 8.314×10^{-3} | $\text{kJ K}^{-1} \text{mol}^{-1}$ |
| | | | T | 298.15 | K |
| | | | F | 96.49 | kC mol^{-1} |

Data sources for formation energies and binding polynomials

To calculate the ΔG of a biochemical reaction using the binding polynomials requires input data on the binding polynomials, P_j (which we can calculate from dissociation constants, K_k , of all the species), and the standard Gibbs energy of formation $\Delta_f G^0$ for the reference species under defined state conditions (temperature, pressure, ionic strength). Using Alberty's Legendre transform formalism we require standard Gibbs energies of formation for all the species of each reactant.

To obtain these values we can refer to databases such as the database developed by Alberty (1). This database contains the standard Gibbs energies of formation for species ($\Delta_f G_i^0$), and dissociation constants (K_k) needed to calculate the binding polynomials (P_j). The Gibbs energies of formation in this database are derived from experimentally determined equilibrium constants that were obtained from various literature sources. Because Alberty's database contains a limited amount of information, efforts to expand

and make this information more complete are being taken (2) and, in fact, ultimately more experiments need to be done.

However, the amount of experimentally determined data is not sufficient for analysis of complete genome-scale metabolic networks. To achieve a more complete analysis of such networks, thermodynamic information can be estimated using the group contribution method (3). Using this method the standard Gibbs energy of formation at zero ionic strength, $\Delta_f G_i^0$, of the most predominant species at pH 7 may be calculated. The estimated Gibbs energies of formation are used with Eq. 1 from the main text to perform thermodynamic analyses of genome-scale metabolic networks. The possibility to do thermodynamic analyses on the genome-scale with estimated formation energies, however, comes at the price of reduced accuracy. In case a more detailed analysis should be performed with these values, i.e. using the formulations that we proposed in Eq. 6 and Eq. 7 from the main text, then we must calculate the $\Delta_f G_i^0$ values for all the species we want to consider of each reactant using dissociation constants of all the species (1).

Correction of Gibbs energies of formation for ionic strength

Under either of the two equivalent concepts outlined above, the value of $\Delta_f G_i^0$ (or $\Delta_f G_{i_{\text{ref}}}^0$) depends on ionic strength in the solution. To account for ionic screening, we can apply the extended Debye-Hückel equation (cf. 4):

$$\Delta_f G_i^0(I) = \Delta_f G_i^0(I = 0) - RT \frac{Az_i^2 I^{1/2}}{1 + BI^{1/2}}, \quad (\text{A4y})$$

where $\Delta_f G_i^0(I = 0)$ is the standard Gibbs energy of formation at ionic strength of 0, z_i is the charge of species i , B is $1.6 \text{ M}^{-1/2}$ and A is a parameter that varies with temperature approximately according to (5):

$$A = 1.10708 - (1.54508 \times 10^{-3})T + (5.95584 \times 10^{-6})T^2. \quad (\text{A4z})$$

The dependence of cation binding dissociation constants on ionic strength may also be accounted for using the extended Debye-Hückel equation (2). Additionally, the dependence of reaction Gibbs energies and dissociation constants on temperature may be accounted for when and if the corresponding enthalpies are known.

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